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Short communication

CYP3A4 inhibition by *Psoralea corylifolia* and its major components in human recombinant enzyme, differentiated human hepatoma HuH-7 and HepaRG cells

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ABSTRACT

Psoralea corylifolia (*P. corylifolia*) is a medicinal plant used primarily in herbal dietary supplements to treat skin diseases, such as vitiligo and psoriasis. Case reports of liver toxicity have recently emerged from its use, which often includes co-administration with other herbal products. In this study, CYP3A4 inhibition and hepatotoxicity of *P. corylifolia* and its major components were evaluated in human recombinant CYP3A4 enzyme, differentiated human hepatoma HuH-7 and HepaRG cells. LC/MS-TOF was used to identify the major components of *P. corylifolia* fruit methanol–water extract. *P. corylifolia* and its major bioactive components psoralen and isopsoralen were then incubated with human recombinant CYP3A4 (10 min) or differentiated HuH-7 and HepaRG cells (24 h) prior to CYP3A4 activity and cytotoxicity assays. *P. corylifolia* extract, psoralen, and isopsoralen concentration dependently inhibited CYP3A4 activity with different potency in the three *in vitro* systems. No cytotoxicity was observed at any concentration tested. *In vitro* CYP3A4 inhibition by *P. corylifolia* and its major components suggests potential drug–dietary supplement interactions that warrant further investigations *in vivo*.

1. Introduction

The medicinal plant *Psoralea corylifolia* (*P. corylifolia*; Babchi) is widely distributed and used as a natural alternative remedy due to its diverse bioactivities, including hepatoprotective, estrogenic, antidepressant, antimicrobial, antioxidant, and antitumor effects [1]. However, a few case reports have shown hepatotoxicity associated with *P. corylifolia* administration. In one case study, a 44-year-old female ingested *P. corylifolia* seeds every 1 h for 7 weeks for treating osteoporosis and developed acute cholestatic hepatitis [2]. In another case study, a 64-year-old female developed severe hepatotoxicity after 9 months administration of three kinds of herbal tablets and an herbal tea for treating vitiligo. Tablets made from *P. corylifolia* leaves were identified as the most probable cause of the liver toxicity [3]. Furthermore, there were three cases of liver injury, including acute hepatitis, jaundice or abnormal liver biochemistry, associated with consumption of *P. corylifolia* dried seeds (*Fructus psoraleae*) in the Chinese population [4]. Psoralen and isopsoralen are the two major furocoumarins in *P. corylifolia.* Psoralen plus UVA (PUVA) therapy has been used to treat skin diseases, such as vitiligo and psoriasis [5]. Long-term psoralen or isopsoralen usage has been associated with hepatotoxicity in mice and rats [6,7]. However, the underlying hepatotoxic mechanisms of *P. corylifolia* and its major components are not well elucidated.

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Cytochrome P450 3A4 (CYP3A4) is the most abundant liver and intestine metabolic enzyme, and it plays a key role in the detoxification and bioactivation of drugs and herbal dietary supplements [8]. CYP3A4 mediated liver toxicity together with drug–drug and drug–dietary supplement interactions are major concerns in drug development and consumer safety [9]. Although CYP3A4 inhibition or induction does not directly cause hepatotoxicity, the subsequent interactions of co-administered substances could lead to over-dose and toxicity such as that reported for St. John's wort [10]. Subcellular fractions like human recombinant enzyme and microsomes lack an intact cellular system and often overestimate CYP3A4 inhibition [11]. Therefore, primary human hepatocytes or metabolically competent hepatoma cell lines, such as dimethyl sulfoxide (DMSO) treated HuH-7 cells [12] and HepaRG cells [13], serve as additional tools for investigating CYP3A4 activity *in vitro*. DMSO-treated HuH-7 cells express hepatocyte differentiation marker genes, elevated cytochrome P450s and glucuronosyltransferases [12], making them a valuable model for *in vitro* enzyme inhibition and hepatotoxicity studies. HepaRG cells express comparable or higher levels of metabolic enzymes, especially CYP3A4, compared to primary human hepatocytes [14,15].

In this study, CYP3A4 inhibition and hepatotoxicity of *P. corylifolia*, and its bioactive components psoralen and isopsoralen, were investigated using human recombinant CYP3A4 enzyme, DMSO-treated HuH-7 and HepaRG cells. Troleandomycin, a potent CYP3A4 mechanism-based inhibitor [8], was used as a positive control in the *in vitro* systems.

2. Materials and methods

2.1. Materials

Human hepatoma HuH-7 cells were obtained from Health Science Research Resources Bank, Japan Health Sciences Foundation (Osaka, Japan). HepaRG cells were purchased from Life Technologies (Grand Island, NY). Cell culture medium and medium supplements were purchased from Life Technologies (Grand Island, NY). Fetal bovine serum was purchased from Atlanta Biologicals (Lawrenceville, GA). The following compounds (all ≥97% purity) were obtained from Sigma–Aldrich (St. Louis, MO): isopsoralen, DMSO, troleandomycin, methanol, acetonitrile, water, and formic acid. P450-Glo CYP3A4 assay (luciferin-IPA) and CellTiter-Fluor cell viability assays were purchased from Promega (Madison, WI). Supersomes human CYP3A4 plus reductase and b5, NADPH regenerating system, and rat tail collagen type I were obtained from Corning (Corning, NY). Authenticated *P. corylifolia* fruit was purchased from American Herbal Pharmacopoeia (Scotts Valley, CA). Psoralen (≥98%) was obtained from Enzo Life Sciences (Farmingdale, NY).

2.2. Preparation of P. corylifolia fruit extract

P. corylifolia fruit was ground to a fine powder using a pestle and mortar. The powder was extracted with 10 mL methanol–water (1:1) per gram. The mixture was sonicated in a water bath for 30 min at room temperature followed by centrifugation at 1000 × *g* for 5 min. The supernatant was filtered through a 0.45 μm filter into a glass vial, and solvent was removed using a SpeedVac evaporator. Dry samples were stored at 4 °C protected from light.

2.3. LC/MS-TOF identification of major components of P. corylifolia fruit extract

P. corylifolia fruit extract was dissolved to a final concentration of 100 μg/mL in acetonitrile–water (5:95). Compounds were separated on a 50 mm × 3.0 mm (2.7-micron) Poroshell 120 EC-C18 column (Agilent Technologies, Santa Clara, CA) eluted with a 9 min gradient of 30–98% of solvent B at a flow rate of 0.5 mL/min. Solvent A was composed of 0.1% formic acid in water; while solvent B was composed of 0.1% formic acid in acetonitrile. Column effluent was analyzed in a 6520B QTOF hybrid mass spectrometer (Agilent Technologies, Santa Clara, CA). Data were collected in positive ESI mode. For MS/MS, collision cell energy was set to a constant 20 eV. Molecular features were identified from total ion chromatograms (TIC) using Agilent Mass Hunter software. Compounds were tentatively identified by exact mass using both published data sources and the Agilent personal Metlin database with a mass error tolerance of 10 ppm.

2.4. Cell culture

HuH-7 cells were cultured in Dulbecco's minimal essential medium (DMEM) (low glucose, pyruvate, GlutaMax), supplemented with MEM non-essential amino acids (1%), HEPES (10 mM) and fetal bovine serum (10%). HuH-7 cells were plated (6×10^4 cells/cm^2) in the 96-well tissue culture plates pre-coated with rat tail collagen type I. HuH-7 cells were first cultured to confluence (1 week), and then treated with 1% DMSO (2 weeks) before use.

HepaRG cells were grown according to the supplier's protocol, and seeded in 96-well plates (3×10^5 cells/cm^2) pre-coated with rat tail collagen type I. Four hours after plating, HepaRG cells were used for CYP3A4 inhibition and hepatotoxicity studies.

2.5. Measurement of CYP3A4 inhibition

For cell-based studies, DMSO-treated HuH-7 and HepaRG cells were incubated with *P. corylifolia* extract (0.5–100 μg/mL), psoralen (1–200 μM), isopsoralen (1–200 μM) or troleandomycin (1–100 μM) at 37 °C for 24 h. Before CYP3A4 activity measurement, treatments were removed, and cells were washed to minimize potential interference from treatment solutions. CYP3A4 activity was determined by incubation with luciferin-IPA (3 μM; K_m value) for 30 or 60 min. The reaction was terminated by addition of luciferin detection reagent, followed by luminescence measurement.

For human recombinant CYP3A4 enzyme inhibition studies, the incubation mixture (100 μL) consisted of the following: potassium phosphate buffer (0.1 M, pH 7.4), NADPH regenerating system, human recombinant CYP3A4 (0.1 pmol), luciferin-IPA (3 μM; K_m value), and *P.*

corylifolia extract (0.5–80 μg/mL), psoralen or isopsoralen (1–200 μM), or troleandomycin (0.02–1 μM). The reaction was initiated by adding human recombinant CYP3A4 and terminated by addition of the luciferin detection reagent after a 10 min reaction at room temperature. Luminescence readings of *P. corylifolia* extract, psoralen, isopsoralen and troleandomycin treatments were used for background correction when calculating CYP3A4 inhibition.

2.6. Measurement of cytotoxicity

Cell viability (live-cell protease activity) of DMSO-treated HuH-7 and HepaRG cells after CYP3A4 assay was determined using the CellTiter-Fluor assay. Cells were incubated with CellTiter-Fluor substrate glycylphenylalanyl–aminofluorocoumarin (GF-AFC) at 37 °C for 60 min before fluorescence measurement.

2.7. Data analysis

SigmaPlot 8.0 (San Jose, CA) was used to fit CYP3A4 inhibition data and calculate IC_{50} values. IC_{50} was the half-maximal inhibitory concentration of psoralen, isopsoralen, *P. corylifolia* or troleandomycin determined using a Hill nonlinear regression model.

3. Results and discussion

3.1. LC/MS-TOF characterization

Fig. 1A displays the extracted compound LC/MS chromatogram of *P. corylifolia* fruit extract. The chromatogram is qualitatively similar to a published LC fingerprint for *P. corylifolia* fruit [16], with psoralen and isopsoralen comprising 41% and 22% of the total peak volume, respectively. MS/MS analysis using authentic standards (Fig. 1B and C) confirmed the identities of psoralen and isopsoralen in the methanol–water extract of *P. corylifolia* fruit.

3.2. Measurement of cytotoxicity

No significant cytotoxicity (<15%) was observed in DMSO-treated HuH-7 and HepaRG cells at any concentration tested after 24 h treatment.

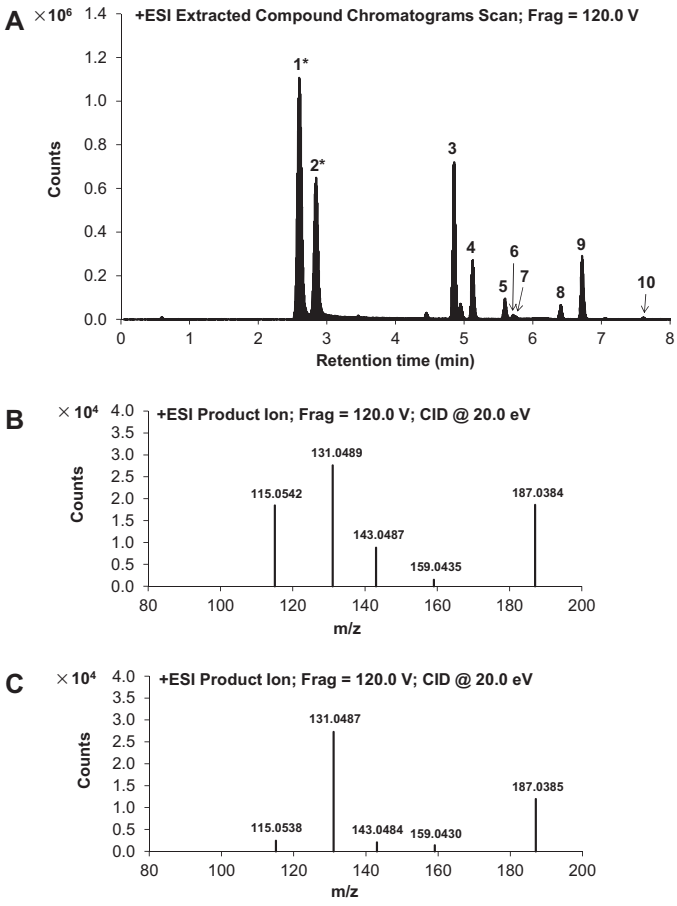

Fig. 1. (A) Total extracted compound chromatogram from LC/MS-TOF analysis of *Psoralea corylifolia* fruit extract. Compound identifications were made putatively by retention time and exact mass except those marked by '*' which were confirmed using authentic standards: (1) psoralen*; (2) isopsoralen*; (3) neobavaisoflavone; (4) bavachin; (5) corylin; (6) bavachromene; (7) psoralidin*; (8) isobavachalcone; (9) bavachinin; (10) bavachalcone. (B and C) MS/MS spectra of psoralen and isopsoralen peaks, respectively. Retention times and MS/MS spectra were identical to those of authentic standards (data not shown).

Table 1
IC_{50} values of CYP3A4 in different *in vitro* systems.

	Human recombinant CYP3A4	DMSO-treated HuH-7	HepaRG[a]
Psoralen (μM)	30.8	57.7	>200
Isopsoralen (μM)	36.8	29.1	>200
P. corylifolia (μg/mL)	6.0	20.6	>100
Troleandomycin (μM)	0.1	27.8	0.6

[a] Less than 30% of CYP3A4 inhibition was observed in HepaRG cells for psoralen, isopsoralen or *P. corylifolia* up to the highest concentration tested.

3.3. Measurement of CYP3A4 inhibition

For cell-based studies, CYP3A4 activity was normalized by cell viability for individual samples. Psoralen, isopsoralen, and *P. corylifolia* concentration dependently inhibited CYP3A4 activity, yielding IC_{50} values (Table 1) varying within 3.5 fold between human recombinant CYP3A4 enzymes and DMSO-treated HuH-7 cells. However, psoralen, isopsoralen, or *P. corylifolia* inhibited less than 30% of CYP3A4 activity (data not shown) in HepaRG cells up to the highest concentration tested.

Observed differences between human recombinant CYP3A4 enzyme, DMSO-treated HuH-7 cells, and HepaRG cells could result from the physical-chemical properties of test substances, differential nonspecific binding, cell membrane permeability, active transport, or involvement of additional metabolic enzymes. Furthermore, it is known that the basal CYP3A4 activity in different cell systems ranks in the order of HepaRG cells > primary human hepatocytes > DMSO-treated HuH-7 cells [12,14]. Minimal inhibition of CYP3A4 by psoralen, isopsoralen, and *P. corylifolia* could also result from the extensive metabolism and efflux transportation in HepaRG cells, which express comparable or higher levels of various metabolic enzymes and efflux transporters compared with primary human hepatocytes [17]. These results demonstrate the value of using multiple *in vitro* systems when studying enzyme inhibition.

CYP3A4 inhibition by *P. corylifolia* is important because *P. corylifolia* is often co-administered with drugs or other dietary supplements [7]. Once CYP3A4 is inhibited, the metabolism of substrate drugs or dietary supplements would be compromised and cause over-dose or toxicity. Clinical cases of *P. corylifolia* toxicity often involve co-administration of multiple herbal drugs [3]. Thus, toxicity attributed to *P. corylifolia* could arise from CYP3A4 inhibition and subsequent elevated levels of co-administered CYP3A4 substrate drugs or dietary supplements. In the current study, *P. corylifolia* fruit extract and its two most abundant components psoralen and isopsoralen were studied for CYP3A4 inhibition, but other components could also contribute to overall inhibition.

It is critical that test agent concentrations used in *in vitro* studies are relevant to *in vivo* exposure. A pharmacokinetic study of oral administration of *P. corylifolia* fruit (9.12 mg/kg coumarin components solution containing 31.26% psoralen and 48.13% isopsoralen) in rats reported plasma C_{max} of 135 μM and 201 μM for psoralen and isopsoralen, respectively [18]. Since the concentrations used in the current study are comparable to *in vivo* concentrations for psoralen and isopsoralen, our *in vitro* CYP3A4 inhibition data is relevant to *in vivo* situations. However, it is important to point out that potential species differences may play a role in the *in vivo* pharmacokinetics of *P. corylifolia* and related compounds between lab animals and human.

The inhibitory potency of psoralen and isopsoralen was compared to troleandomycin, a strong CYP3A4 irreversible inhibitor *in vitro* [19]. Different rankings of CYP3A4 inhibition potency were observed in the three *in vitro* systems (Table 1). It is worth pointing out that *in vitro* inhibition does not necessarily translate to *in vivo* CYP3A4 inhibition because of differential absorption, efflux transportation, and first-pass metabolism, which all contribute to the presystemic removal of xenobiotics. Therefore, the clinical relevance of the current results warrants further evaluation *in vivo*.

4. Conclusion

P. corylifolia and its major bioactive components psoralen and isopsoralen are *in vitro* CYP3A4 inhibitors. Animal and clinical interaction studies are needed for further validation. Caution may be needed when co-administering *P. corylifolia* with other herbals or drugs which are CYP3A4 substrates.

Transparency document

The Transparency document associated with this article can be found in the online version.

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